tension sensitive than other myosins with similar length lever arms, suggesting that different myosins have different tension-sensitive transitions.

2896-Pos
Myosin-I Dependent Membrane-Cytoskeleton Adhesion as a Regulator of Cell Surface Morphology
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All cellular functions involving deformation of the plasma membrane (e.g. endocytosis, exocytosis, the formation of surface protrusions) are regulated by the apparent membrane tension (T_m), a composite of the surface tension (σ) and the adhesion provided by molecules linking membrane to the actin cytoskeleton (γ). Using an optical trap based tether force assay, we recently demonstrated that class I myosins, a family of membrane-binding actin-based motor proteins, control membrane tension by mediating membrane-cytoskeleton adhesion. More specifically, these studies revealed that the membrane-cytoskeleton adhesion provided by myosin-I increases apparent membrane tension as indicated by the force required to pull a single membrane tether from the cell surface. Interestingly, the physical links to the cytoskeleton provided by myosin-I also allow the cell to form multiple adjacent tethers. However, when multiple tethers are pulled, tether lifetimes appear to decrease as apparent membrane tension increases. Here we present a thermodynamic model, which accounts for the impact of apparent membrane tension on the lifetime of multiple tethers. We argue that, in the context of our experimental geometry, the global increase in apparent membrane tension that results from greater membrane-cytoskeleton adhesion, works locally to accelerate the rupture of bonds between the membrane and cytoskeleton, which would otherwise prevent individual membrane tethers from coalescing. We also elaborate on this concept to develop a separate model, which shows that tuning the level of membrane-cytoskeleton adhesion may enable cells to vary the density of surface protrusions (i.e. # of structures per unit area membrane). As such, myosin-I dependent membrane-cytoskeleton adhesion emerges as the key regulator of cell surface morphology.

2897-Pos
Kinetics of Myosin-I-Membrane Detachment Under Load
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Myo1c is a single-headed motor that links cell membranes to the underlying actin cytoskeleton. Actin binding occurs via the motor domain, while the tail domain interacts with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) through a putative pleckstrin homology (PH) domain. In this study, we measured the strength and lifetime of the myo1c attachment to spherical supported lipid bilayers (SSL) composed of 1 μm diameter silica or polystyrene beads coated with various mole fractions of PI(4,5)P2, dioleoyl-phosphatidylcholine (DOPC), and dioleoyl-phosphatidylserine (DOPS). The SSLs trapped by a laser beam were brought into contact with immobilized spherical (2 μm) silica pedestals sparsely labeled with the myo1c tail domain. Upon repeated contact and retraction cycles, binding events and subsequent rupture forces were measured. The most probable rupture forces (< 10 pN) from membranes containing 2% PI(4,5)P2 were found to be largely independent of the loading rate (pN/s). Nevertheless, the frequency of single myo1c molecules interacting with membranes containing 2% PI(4,5)P2 - 98% DOPC was considerably higher than with membranes that contained 20 - 80% DOPS. Lifetime measurements of myo1c attachment to 2% PI(4,5)P2 - 98% DOPC membranes under constant pulling forces (1 - 3 pN) were fitted to Bell's equation and the extrapolated duration of the bond at zero force was found to be ~ 25 ms. Although phosphoinositide binding is crucial for the proper cellular targeting of myo1c, our results suggest that it is unlikely that this connection is a suitable anchor for force generation.

2898-Pos
Kinetics and Thermodynamics of Nucleotide Binding Pocket Opening/closing in Myosin V Monitored with FRET
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Kinetic and structural studies of both muscle and non-muscle myosins have revealed that the enzymatic cycle of these motors frequently contains more than one actomyosin ADP state. Interestingly, the rate of ADP release in myosin motors is thought to be the main determinant of sliding velocity in muscle, suggesting strain dependent ADP release may be a critical mechanism of mechanochemical coupling. Our previous work has demonstrated that labeling myosin V in the upper 50 kDa domain with the biarsenical dye FlAsH (MV FlAsH) can serve as an acceptor for fluorescence resonance energy transfer studies with mant labeled nucleotides. We also determined that this donor-acceptor pair likely monitors opening/closing of the nucleotide binding pocket. Currently, we utilized the FRET signal to examine the kinetics of nucleotide binding pocket opening during the process of mantADP release from acto-MV FlAsH. We obtained evidence that the nucleotide binding pocket goes from a closed to an open conformation prior to the release of ADP. We also explored the temperature dependence of the closed to open transition and nucleotide release steps. We find that at lower temperatures the closed conformation is favored while at higher temperature the open conformation is favored. The more rapid ADP release step which follows nucleotide binding pocket opening is also temperature dependent. Therefore, since both steps are temperature-dependent they likely require significant conformational changes. We also compared our FRET results to the rate of ATP-induced dissociation from actin in the presence of ADP monitored by light scatter. Understanding how strain alters either of these two steps may be critical for elucidating the structural mechanism of strain-dependent ADP release in myosins.

2899-Pos
Coupling the Actin Binding Cleft and Nucleotide Binding Pocket in Myosin V
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Previously we have demonstrated in fluorescence resonance energy transfer (FRET) studies that mant labeled nucleotides and IAEDANS actin can act as good donor probes for a FlAsH labeled acceptor site in the upper 50 kDa domain of myosin V. We examined the temperature dependence of the FRET signal between mantADP and MV WT FlAsH in the presence and absence of actin. We found that at low temperature (4-15°C) a high FRET state dominates (closed pocket) while at high temperature (30-35°C) a low FRET state dominates (open pocket). This transition is reversible suggesting a temperature-dependent conformational change. However, the mutant E442A, which is incapable of hydrolyzing ATP, remains in a high FRET state (closed pocket) with mantATP bound in the presence or absence of actin. Our results suggest a more flexible conformation of myosin in the presence of ADP compared to ATP which allows myosin to populate two actomyosin.ADP state conformations. These results are supported by the lifetime FRET analysis, and by computational FIRST/FRODA analysis of the intrinsic flexibility found in different x-ray crystal structures. We also plan to explore the temperature dependent conformational dynamics of the actin binding cleft using the IAEDANS actin (donor) and MV FlAsH (acceptor) pair in the presence of ATP, ADP, and absence of nucleotide using steady state and lifetime based FRET measurements. Our results will provide critical insights into the mechanocoupling that may occur between the nucleotide-binding pocket and actin binding cleft in myosin motors.

2900-Pos
Interaction of a Class V Myosin from Budding Yeast with its Adapter Protein
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Like their mammalian counterparts, class V myosins in *S. cerevisiae* (Myo2p and Myo4p) bind to various adapter proteins to target a particular cargo for transport. Myo4p uses the adapter proteins She3p and She2p in order to transport mRNA from the mother cell to the bud. She3p binds to the rod of Myo4p, and prevents it from dimerizing, thus forming a single-headed motor complex (Hodges et al., 2008; Bookwalter et al., 2009). Because the Myo4p/She3p complex is single-headed, the question arises as to whether enough motors can bind to a single She2p to enable continuous cargo transport. The She2p crystal structure suggested that She2p exists as a dimer (Niessing et al., 2004). In contrast, our sedimentation equilibrium measurements of She2p were consistent with formation of a tetramer in solution, in principle allowing for binding of four motor heads. We showed that Myo4p/She3p forms a complex with tetrameric She2p in the absence of mRNA, based on sedimentation velocity experiments and co-purification. Mutation of Ser 120 to Tyr converts She2p to a dimer. The ability of the motor complex to bind to this and other She2p mutants is being tested in order to map the binding interface. Total internal reflection fluorescence microscopy is being used to test whether the native She2p tetramer can bind enough single-headed motors to support continuous movement on actin. The ability of She2p mutants to support correct bud tip localization of *ASH1* mRNA in living yeast cells will also be assessed. These studies will help elucidate how a non-processive single-headed motor can act as a cargo transporter.

2901-Pos
The Mechanical Properties of a Single Myosin V Motor Domain During Gait Motion
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Myosin V takes multiple 36-nm steps along an actin filament by coordinating its two motor domains. The properties of myosin V have been addressed by means of several assays. In particular, optical tweezers techniques and FIONA have contributed widely to enlightening the motion mechanism. However, these two assays cannot be applied when studying the mechanism for coordinated force generation by a single motor domain during motion. For myosin V, optical tweezers assays are restricted to studying the mechanical properties of both motor domains when an optically-trapped bead is attached to the Myosin V tail. On the other hand, although FIONA is capable of observing the motion of a single motor domain during gait motion, it is not capable of revealing the mechanical properties because these studies do not apply external force.

Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V based on the previous report by Block's lab. The DNA linker is used to connect a bead to one of the two motor domains. In this experimental setup, external force by the optical tweezers is applied to a single motor domain directly via the DNA linker during gait motion. By using this measurement system, we succeeded to observe single head behavior while force is applied to it.

2902-Pos

Reconstituting a Native Actin Track for Myosin V Transport

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The budding yeast *S. cerevisiae* is an excellent model system for the study of cargo transport by myosin, given the relative importance of actin cables versus microtubules in this cell. Despite this, no *in vitro* studies have tried to mimic the actin-tropomyosin cables along which the class V myosin Myo2p transports secretory vesicles, vacuoles, mitochondria, and other organelles to the growing bud. We find that Myo2p is non-processive *in vitro*,in agreement with previous results [1-2]. These experiments were, however, performed using chicken skeletal actin, which is only 87% identical to yeast actin. Accordingly, we are investigating if Myo2p behavior changes when the *in vitro* conditions more closely match those found in the yeast cell. Actin cables will be reconstituted *in vitro* from yeast actin, yeast tropomyosin, and the actin bundling protein fimbrin. The effects of yeast versus skeletal actin, of bundled actin versus single filaments, and of the presence of each of the two different tropomysin isoforms will be tested. Myo2p motility, processivity, and actin binding affinity will be assessed with these different tracks. The effect of varying ionic conditions, nucleotide concentration, and viscosity will also be tested, to determine if Myo2p behavior changes as conditions more closely match those of the intracellular milieu.

1. Reck-Peterson et al., *JCB* 153 (2001).
2. Dunn et al., *JCB* 178 (2007).

2903-Pos

Liposomes as Model Cargo for Myosin Va

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Myosin Va (myoVa) is a processive, actin-based motor involved in the transport of membrane bound secretory vesicles and organelles. How multiple motors attached to such cargo generate productive forward motion is unclear. To address this, we have coupled expressed myoVa-HMM with a C-terminal biotin tag to extruded fluorescent, neutravidin-coated liposomes as an in vitro model for intracellular cargo. This model system allows control over liposome size, fluidity, and surface density of attached motors. When observed in TIRF on actin tracks at room temperature, "rigid" 400nm DPPC liposomes with ~160 motors/liposome move at speeds equal to that of a single processive myoVa-HMM (510 ± 227nm/s), whereas, 200nm liposomes with the same surface density of motors move 30% slower (352 ± 121nm/s). In comparison, "softer" 200nm DMPC/cholesterol liposomes that have more fluid phase membranes are slower yet (229 ± 130nm/s). These velocity data suggest a complex relationship between the ensemble of attached motors and the liposome rigidity/fluidity and size. With more fluid membranes, motors may be mobile within the liposome membrane, compromising their contribution to forward motion and thus the slower velocities. In contrast, larger, more rigid liposomes may allow a fixed number of transporters to remain productively engaged. Interestingly, liposomes can be observed "cartwheeling" along actin tracks, suggesting that motors can exchange roles between being an active transporter and a passenger waiting its turn as the liposome effectively rolls down the actin track. By attaching Qdot-labeled myoVa-HMM, the exact spatial relationship between the motor and liposome cargo can be determined to help understand and model the complexities of this simplified in vitro representation of intracellular cargo transport.

2904-Pos

Myosin Va Cargo Transport on Actin Bundles

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Myosin Va (myoVa) walks processively while carrying cargo towards the plus end of actin filaments. In cells, parallel actin filament bundles (e.g. stress fibers and filopodia) present a directional challenge to myoVa cargo transport. Therefore, we formed unipolar (fascin) and mixed polarity (alpha-actinin) actin bundles as tracks for expressed myoVa-HMM with a C-terminal biotin tag. In this assay, a single streptavidin-Qdot served as cargo for one or many (~ 5) myoVa motors. Qdots transported by one or many myoVa molecules traveled in the same direction on unipolar bundles, while moving in either direction on mixed polarity bundles. Qdot speeds were the same regardless of bundle type or number of motors (400nm/s), and similar to that for one or many motors on a single actin filament (Nelson et al., 2009). However, run lengths for single motors were 2-3 times longer on bundles than previously observed on single actin filaments. This suggests that on parallel tracks the leading head has a greater number of actins within its reach, thus decreasing the probability of run termination. Interestingly, on mixed polarity bundles, we observed individual Qdots changing directions in the middle of a run, the frequency of which increases in the multiple motor case. It was not surprising that a Qdot with a single motor can switch directions on a mixed polarity bundle, given myoVa's inherent flexibility that allows it to turn up to 150o at actin filament intersections (Ali et al., 2007). These data also suggest that one or many myoVa molecules bound to a single cargo have the ability to jump tracks to neighboring actin filaments. With Qdot-labeling of the individual heads, high spatial resolution studies will confirm this on mixed polarity bundles, and determine whether the motors also wander on unipolar bundles.

2905-Pos

Flexibility of Stepping Manner of Myosin V and X Processive Movement on 2D Actin Structures

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In a TIRF *in vitro* motility assay, we investigated the processivity and stepping characteristics of myosin V HMM and myosin X HMM with a leucine zipper on single actin filaments and actin bundles. Actin was polymerized and cross-linked on a charged lipid monolayer in Teflon wells in order to create regular 2D or 3D structures. Two cross-linking proteins were used: alpha-actinin which produces non-polarized bundles with 40 nm filament spacing and fimbrin which produces polarized actin bundles with 14 nm filament spacing. We were determined the velocities and the run length for processive movement on those 2-D actin bundles by using modified particle tracking softwear. Myosin V moved processively on all types of in vitro actin structures. Myosin X moved well on polarized fimbrin cross-linked bundles but movement was impaired or non-existent on non-polarized alpha-actinin bundles. Furstermore, we have measured the stepping manner of myosin V and X by using FIONA analysis, which allow us to measured nano-meter precision. Myosin V steps along single actin filaments, while myosin X steps over several actin filaments on the 2-D actin filaments. We hypothesize that forward runs of myosin X on alpha-actinin cross-linked bundles are inhibited because myosin X "sidesteps" to a parallel oppositely polarized filament and the run stalls. The presence of a SAH domain in the lever arm of myosin X could increase the working stroke or flexibility of the lever arm and allow it more easily sidestep the larger alpha-actinin filament spacing.

2906-Pos

Mechanical and Kinetic Properties of a Myosin 5-SAH Chimera

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We have determined the kinetic and motile properties of a myosin 5a HMM construct in which four calmodulin-binding IQ motifs are replaced by the putative single alpha helical domain (SAH) of similar length from Dictyostelium myosin, MyoM. Electron microscopy of this chimera showed that the SAH domain was straight and 17 nm long as predicted, restoring the truncated lever to the length of wild type (Myo5-6IQ). The powerstroke (21.5 nm) measured in